Adalimumab and Infliximab Bind to Fc-receptor and C1q and Generate Immunoprecipitation: A Different Mechanism From Etanercept

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INTRODUCTION

TNF Antagonists

- Tumor necrosis factor (TNF) antagonists have been shown to be efficacious in the treatment of several autoimmune diseases, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and psoriasis
- There are currently 2 classes of biologic drugs that target TNF bioavailability: soluble TNF receptors (etanercept) and anti-TNF monoclonal antibodies (adalimumab and infliximab)
- All 3 currently available agents bear the Fc portion of complement-activating human IgG1; the Fc region is a native component of the monoclonal antibodies, whereas it is genetically fused to the soluble receptor

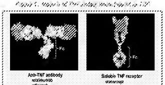
Fc Receptors and TNF Antagonists

- Fc regions bind to Fc receptors (FcγR), which are a family of immunoglobulin-binding molecules expressed on immune cells, including macrophages, granulocytes, natural killer cells, B cells, and platelets
  Members of the FcγR family of receptors are distinguished by the isotype and affinity of the immunoglobulin ligand
- Antibody-dependent cellular cytotoxicity (ADCC) is mediated by cross-linking of FcγR
- Complement-dependent cytotoxicity (CDC) may be enhanced by the cross-linking of FcγR, which increases affinity for the complement component C1q

HYPOTHESIS

- Anti-TNF monoclonal antibodies, but not soluble TNF receptors, form large protein complexes with TNF, resulting in cross-linking of FcγR, and increased binding to C1q, which can lead to ADCC and CDC
- Differences in the ability to activate ADCC and CDC pathways may explain the differences in efficacy in treatment of some diseases (eg, Crohn's disease) and the occurrence of adverse events (eg, granulomatous diseases)

Figure 1. Models of TNF antagonists



TNF antagonists are shown in white, and TNF trimers are blue, green, and red. The TNF-binding site of the anti-TNF antibody is shown in cyan

METHODS

Size-exclusion chromatography–light scattering (SEC-LS)

Samples of individual drugs or mixtures of drugs with different molar ratios of TNF were applied to High Pressure Liquid Chromatography (Agilent 1100) with a column (Superdex-200) to allow separation by size. The samples were then passed through a laser light scattering detector (Wyatt miniDawn) to determine the molecular mass and radius of the protein complexes.

Ouchterlony double-diffusion assays

Samples were applied to the outside wells of a rosette pattern of wells in a flat-bed agarose gel, and control or ligand samples were applied to the center well. Samples were allowed to diffuse through the gel matrix and interact with each other. The gel was stained for proteins. Large protein complexes appeared as dark precipitation lines, whereas stains of small complexes were too faint to visualize

FcγR binding assays

THP 1 cells, a human monocytic cell line expressing 2 types of FcR, FcγRI (CD64) and FcγRII (CD32), were incubated with iodinated drugs under the following conditions:

- Drug alone
- With 0.8-fold molar excess human TNF
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Unbound drug was removed by washing and the amount of cell-bound radiolabeled drug was quantitated in a gamma counter.

C1q binding assays

Human C1q was bound to a 96-well plate via anti-C1q antibodies. Iodinated drugs were added to the plate under the following conditions:

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Unbound drugs were removed by washing, and the C1q-bound radiolabeled drugs were quantitated in a gamma counter.

Binding of TNF antagonists to human whole blood cells

Human whole blood was incubated with iodinated drugs under the following conditions

- Drug alone
- With 0.8-fold molar excess human TNF
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Unbound drug was removed by washing and the amount of cell-bound radiolabeled drug was quantitated in a gamma counter.

RESULTS

Figure 2. SEC Analysis of Etanercept-TNF Complexes

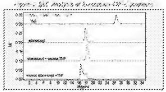


Figure 3. SEC-LS Analysis of Adalimumab-TNF Complexes

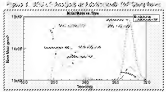


Figure 4. SEC-LS Analysis of Infliximab-TNF Complexes

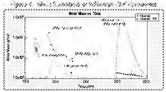


Figure 5. SEC-LS Analysis of Etanercept-TNF Complexes

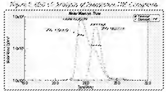
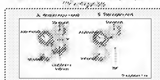


Figure 6. Ouchterlony Double Diffusion Analysis of TNF Antagonists



TNF has been shown to bind as a trimer to both the monoclonal antibodies[1] and the soluble receptor (data not shown). Results from the SEC-LS and Ouchterlony assays predict the following interactions between the 2 classes of drugs and TNF under soluble conditions

Figure 7. Proposed Models of TNF Antagonists and TNF Trimer Complexes

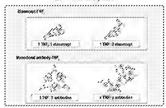


Figure 8. FcγR Binding Assays of TNF Antagonists

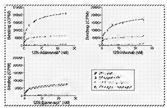


Figure 9. C1q Binding Assays of TNF Antagonists

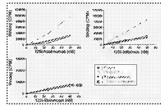


Figure 10. Binding of TNF Antagonists to Human Whole Blood Cells

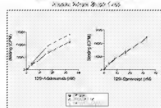
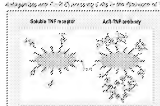


Figure 11. Proposed Models of the Interactions Between TNF Antagonists and FcγR Expressing Cells in the Presence of TNF



DISCUSSION

- Soluble receptors and monoclonal antibodies that target TNF decrease levels of bioactive TNF. Both classes of drugs demonstrate efficacy in the treatment of several autoimmune diseases
- Anti-TNF monoclonal antibodies formed large protein complexes with TNF, whereas the soluble TNF receptor did not
- Formation of large antibody-TNF complexes bound to cells may facilitate cross-linking and activation of cell surface receptors
- All agents bound poorly to FcγR on cells. In the presence of TNF, anti-TNF monoclonal antibodies, but not soluble TNF receptor, increased binding to FcγR
- The ability to bind and cross-link FcγR potentially enables the anti-TNF antibodies to activate ADCC pathways
- Anti-TNF monoclonal antibodies, but not the soluble TNF receptor, bound to the complement component C1q in the presence of TNF
- The ability to bind C1q may confer the ability to activate CDC pathways by TNF-anti-TNF antibody immune complexes
- The molecular mechanisms of cytotoxicity are unclear; evidence for[2] and against[3] ADCC and CDC effector mechanisms by the anti-TNF monoclonal antibody infliximab have been demonstrated
- In the presence of TNF, anti-TNF monoclonal antibodies, but not the soluble TNF receptor, bound to human whole blood cells

CONCLUSIONS

- These differences in binding to FcγR and C1q may lead to differences in immunologic mechanisms, and explain the varying disease states for which these agents are effective treatments. For example, in contrast to the soluble TNF receptor etanercept, the anti-TNF monoclonal antibody infliximab is effective in the treatment of Crohn's disease
- Furthermore, these differences may contribute to the higher rates of fungal and granulomatous infections, such as tuberculosis, observed with infliximab compared with etanercept[4,5]

REFERENCES

1. Santora LC, Kaymakcalan Z, Sakorafas P, Krull IS, Grant K. Characterization of noncovalent complexes of recombinant human monoclonal antibody and antigen using cation exchange, size exclusion chromatography, and BIAcore. Anal Biochem. 2001;299:119-129
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EXHIBIT L

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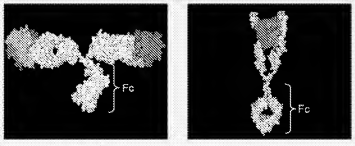

Anti-TNF antibody
adalimumab
infliximab

Soluble TNF receptor
etanercept

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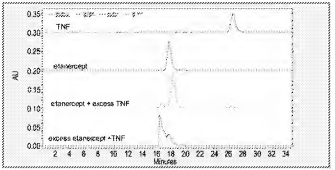

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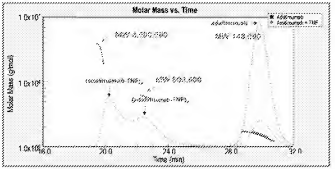

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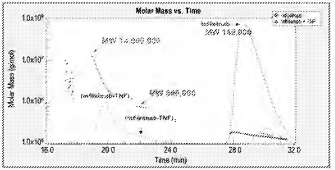

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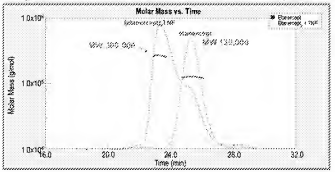

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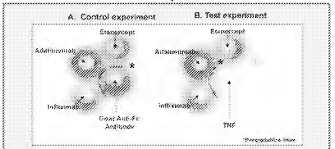

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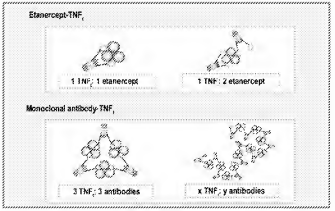

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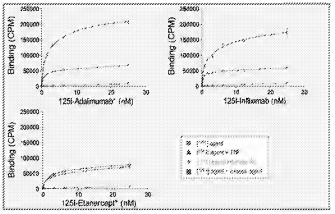

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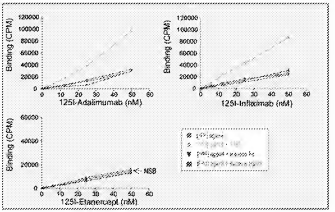

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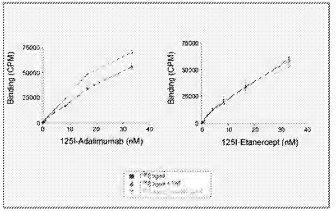

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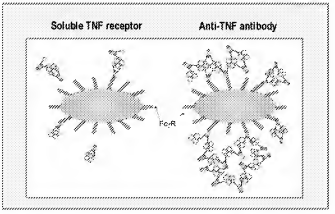

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